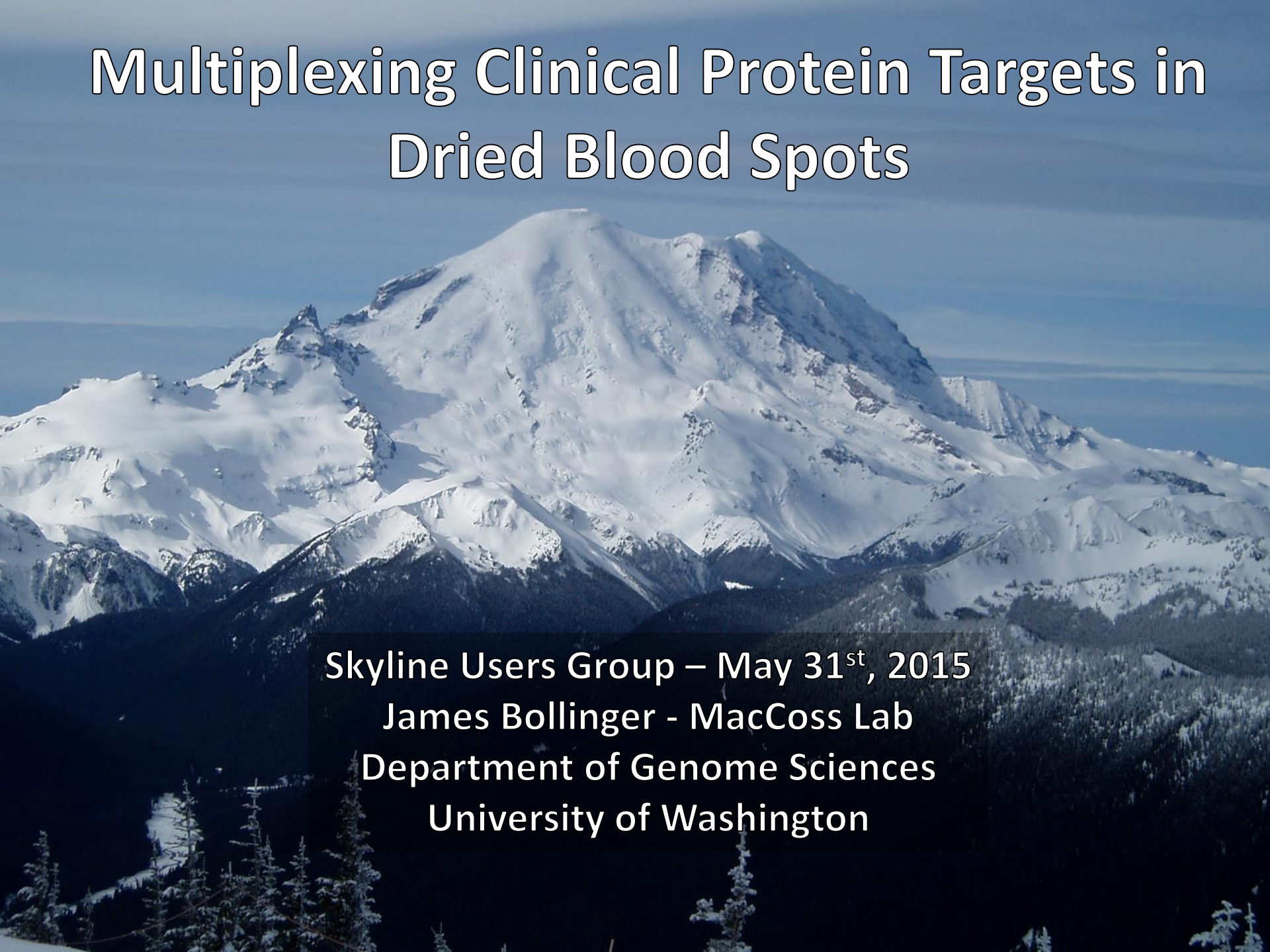
Multiplexing Clinical Protein Targets in Dried Blood Spots



Skyline Users Group – May 31st, 2015

James Bollinger - MacCoss Lab
Department of Genome Sciences
University of Washington

Acknowledgements……..

MacCoss Lab

Mike MacCoss

Brendan Maclean

Rich Johnson

Jarrett Egertson

Genn Merrihew

Sonia Ting

Han-Yin Yang

Scott Goulding

Brook Nunn

Emma Timmons-Schiff

Nick Shulman

Vagisha Sharma

Don Marsh

Brian Pratt

Kaipo Temura

Max Gelb

Hoofnagle Lab

Andy Hoofnagle

Clark Henderson

Jennifer Wallace

Jess Becker

Funding

NIH/NRSA Research Training Grant #T32HG00003

Outline

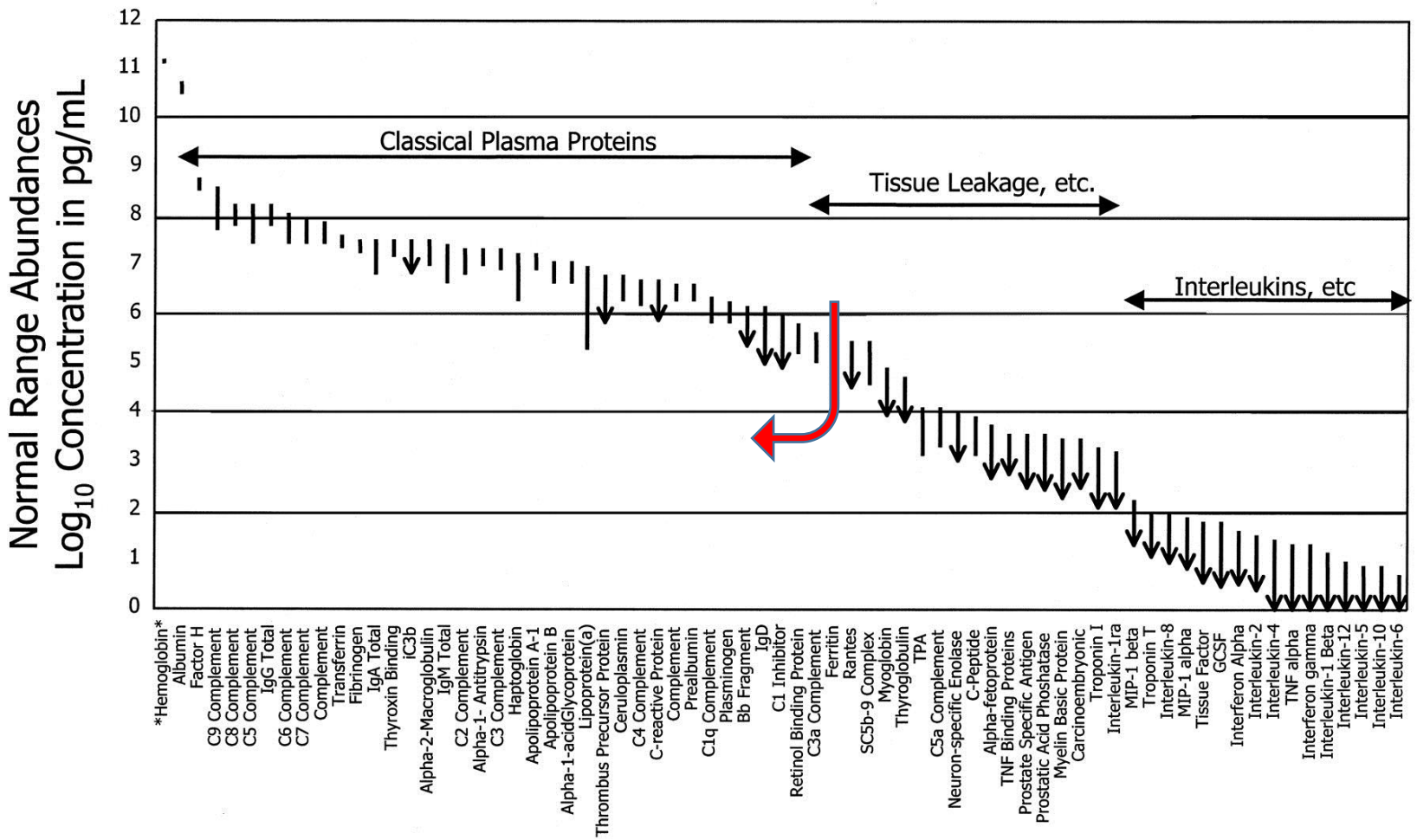
- Selection of candidate proteins for the multiplex analysis of DBS via targeted proteomics
- The currently employed strategies for the selection of candidate peptides for targeted proteomics
- An empirical refinement process for the selection of optimal peptides and their respective MS/MS transitions

Selection of Protein Targets for DBS Wellness Assay

- Acid Phosphatase
- Alanine Aminotransferase
- Albumin
- Aldolase
- Alkaline Phosphatase
- α-1-acid glycoprotein
- α -1-antitrypsin (SERPINA1)
- α -1-antichymotrypsin (SERPINA3)*
- α -1-antiplasmin (SERPINF2)
- α -2-HS-glycoprotein (FetuinA)
- α –fetoprotein (AFP)
- Amylase
- ACE
- Antithrombin III (SERPINC1)
- Apolipoprotein A1
- Apolipoprotein B
- Apolipoprotein C2*
- Apolipoprotein C3*
- Apolipoprotein E*
- Apolipoprotein H*
- Apolipoprotein J*
- Aspartate Aminotransferase
- β -2-microglobulin
- β -Thromboglobulin
- Biotinidase
- Cancer antigen 125
- Cancer antigen 15-3
- Human epididymis protein 4
- Carcinoembryonic antigen (CEA)
- Ceruloplasmin
- Cholinesterase
- Complement C1 (C1R/C1S)
- Complement C1 inhib.
- Complement C1Q
- Complement C3
- Complement C4
- Complement C5
- C-reactive Protein
- Creatine Kinase-BB
- Creatine Kinase-MM
- Cystatin C
- Erythopoeitin
- Factor IX antigen
- Factor X
- Factor XIII
- Ferritin
- Fibrinogen
- Fibronectin
- FSH
- γ glutamyl transferase (I/V)
- Haptoglobin
- β-hCG
- Hemoglobin A1C (HBB)
- Hemopexin
- Her-2/neu
- Human growth hormone
- Human placental lactogen
- IgA
- IgD
- IgE
- IgG
- IgM
- Inhibin-A
- Insulin
- IGF-1
- IGF-2
- IGFBP-1
- IGFBP-2
- Interleukin-2 receptor
- Isocitric dehydrogenase
- κ light chains
- Kininogen 1*
- Lactate Dehydrogenase (heart)
- Lactoferrin
- λ Light Chains
- Lipase
- Lp(a)
- LP-PLA2
- Leutinizing hormone
- Lysozyme
- Myeloperoxidase
- Myoglobin
- Osteocalcin
- Parathyroid hormone
- Phosphohexose isomerase (GPI)
- Plasminogen
- Plasminogen activator inhib.
- Prealbumin (transthyretin)
- BNP
- Procalcitonin
- Prolactin
- Properidin Factor B (CFB)
- Prostatic acid phosphatase
- PSA
- Protein C
- Protein S
- Pseudocholinesterase
- Pyruvate kinase
- Renin
- Retinol binding protein
- Mesothelin-related peptide
- Sorbital dehydrogenase
- Thyroglobulin
- TSH
- Thyroxine binding globulin
- Tissue plasminogen activator
- Transferrin
- Troponin T (TnT)
- Troponin I (TnI)
- Trypsin
- Urokinase
- Vitamin D binding protein*
- Vitronectin
- Von Willebrand factor
- Zinc- α -2-glycoprotein

Adapted from: Tables 1&2 - N. Leigh Anderson. *Clin. Chem.* (2010), 56, 177-185.

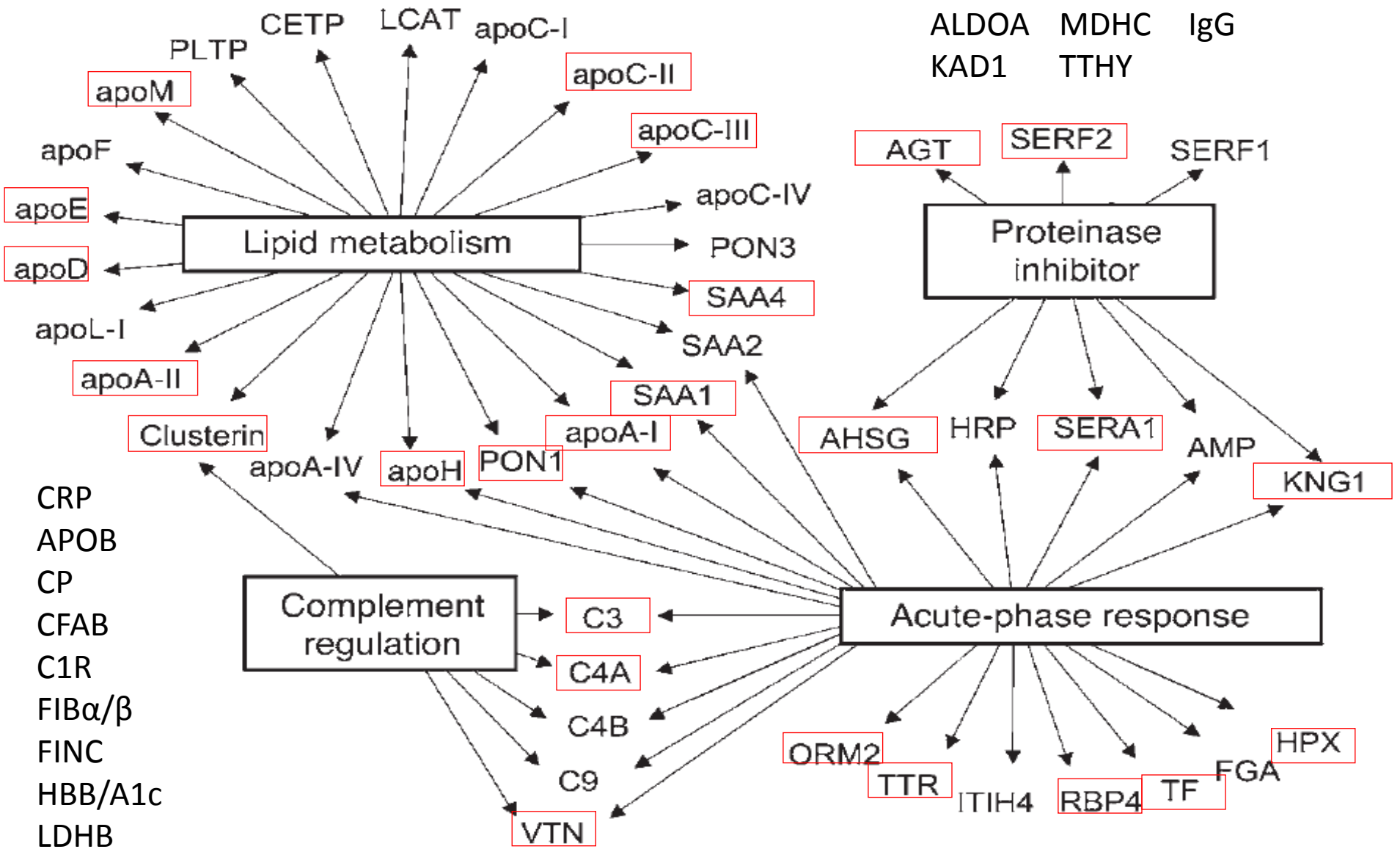
Selection of Protein Targets for DBS Wellness Assay



N. Leigh Anderson & Norman G. Anderson *Mol Cell Proteomics (*2002), 1, 845-867

Selection of Protein Targets for DBS Wellness Assay

TALDO ZA2G VTDB
ALDOA MDHC IgG
KAD1 TTHY

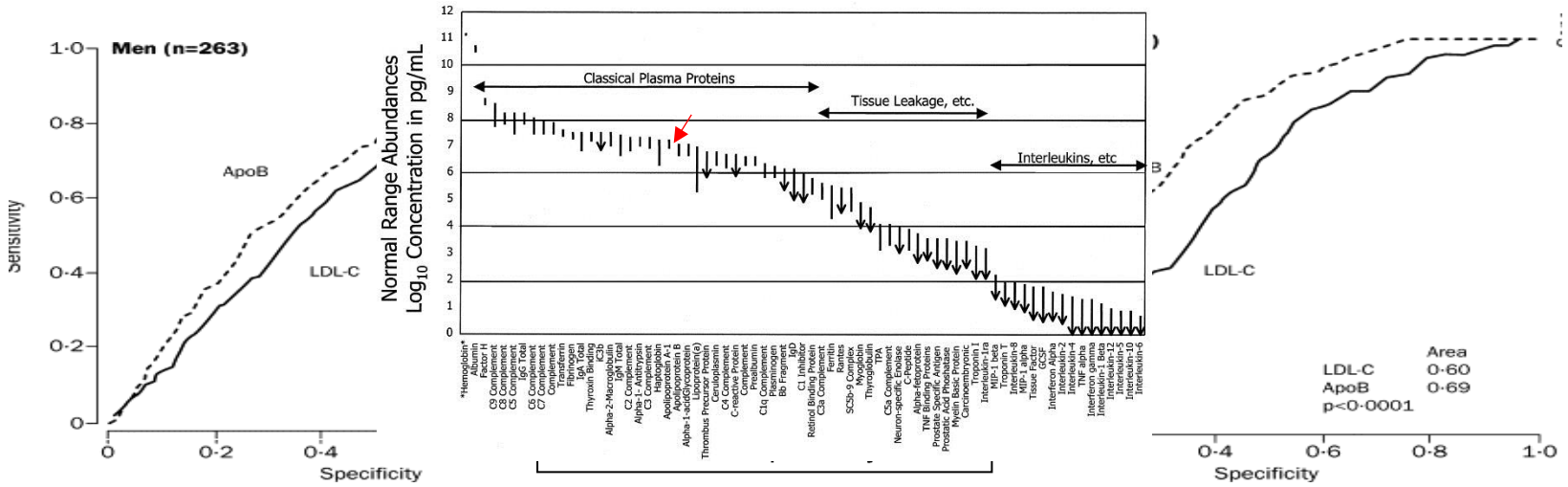


CRP
APOB
CP
CFAB
C1R
FIBα/β
FINC
HBB/A1c
LDHB
SYUA

Adapted from: Vaisar, T. et al., *J. Clin. Inves.* **2007** 117(3); 746–756

How Does One Select an Optimal Set of Peptides for a Targeted Proteomic Experiment?

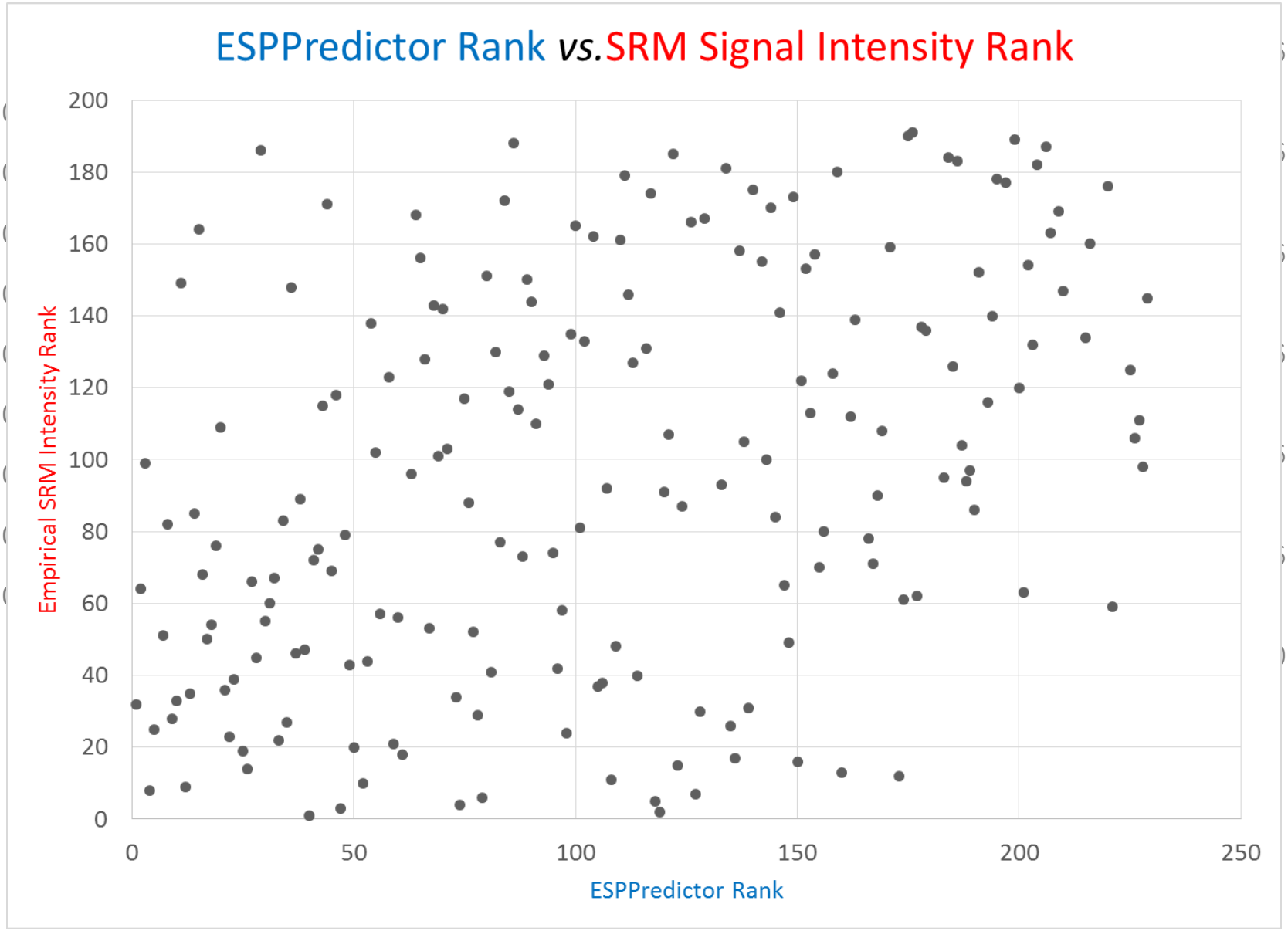
Apolipoprotein B (P04114) – 516 kDa, Serum reference range (~0.5-2 g/L)



Walldius, G. et al. *J Intern Med*. **2006**, 259; 493-519

Walldius, G. et al. *Lancet,* ***2001***, 358(9298); 2026-33

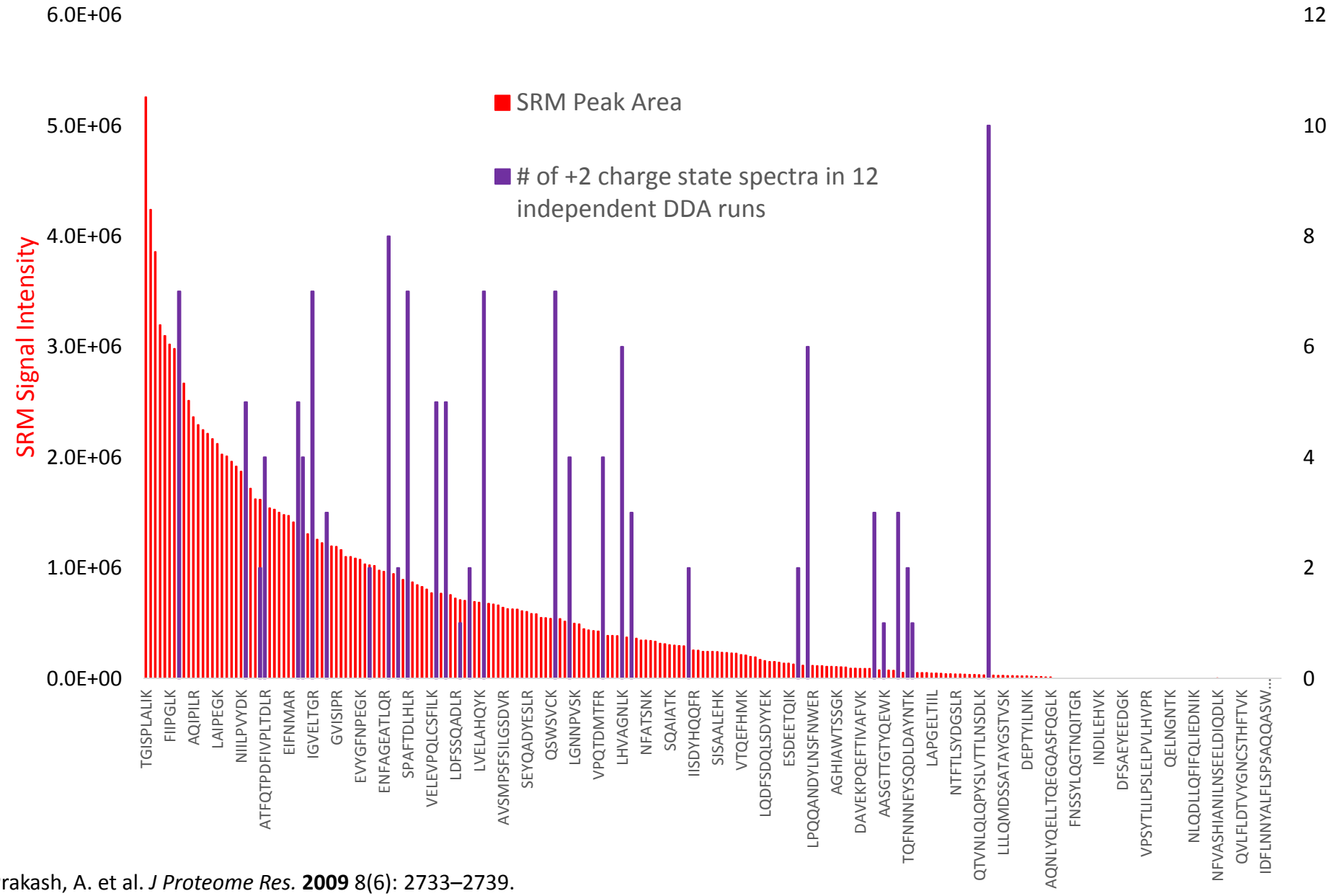
ESPPredictor Score *vs.* SRM Signal Intensity: Apolipoprotein B100



Mallick, P. & Aebersold, R. et al. *Nature Biotechnology*(2007) 25 (1): 125-131

Fusaro, V.A. & Carr, S.A. et al. *Nature Biotechnology* (2009) 27:190-198.

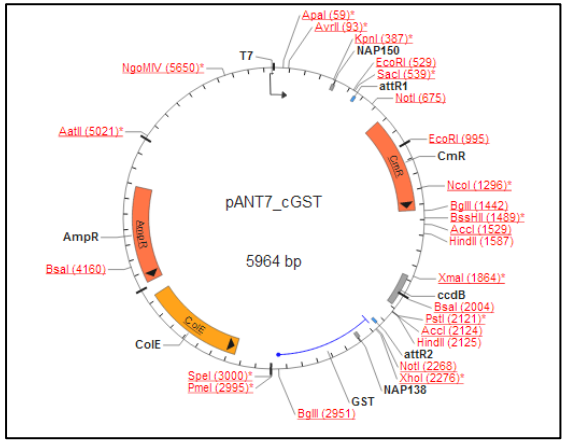
Spectrum Counts *vs.* SRM Signal Intensity: Apolipoprotein B100



Prakash, A. et al. *J Proteome Res.* **2009** 8(6): 2733–2739.

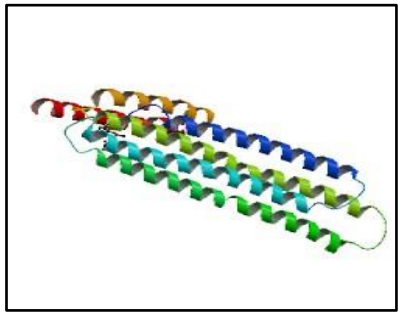
Our Approach……

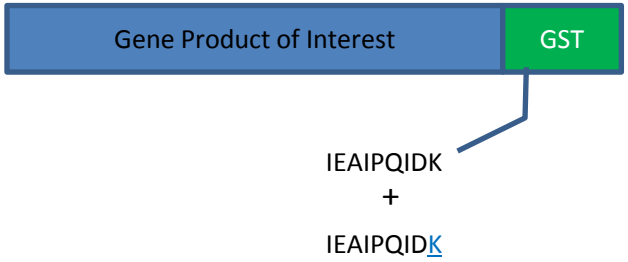
Workup of Recombinant/Native Protein Standard

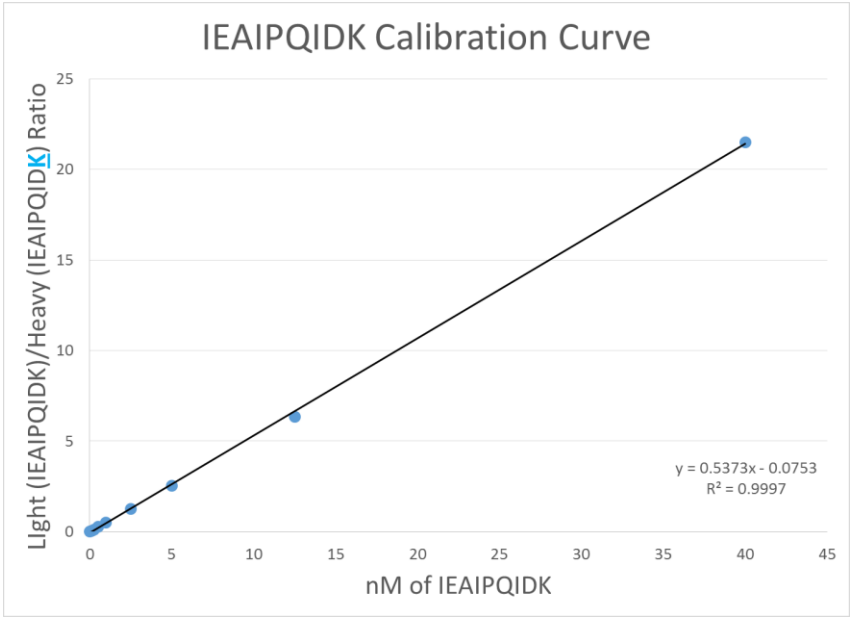


http://dnasu.org

Expand, Purify, & Sequence cDNA clone → ***In Vitro* Transcription and Translation** → **Enrichment via Glutathione-Sepharose Resin**





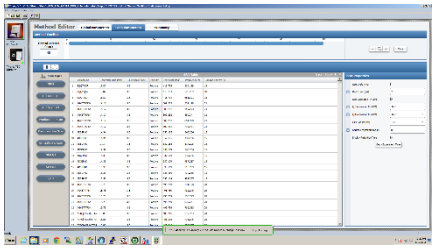


~25-500 femtomoles/25uL IVT

< $20.00/Protein

SRM Method Refinement Cycle

Insert Hypothesis Here



Build SRM Method

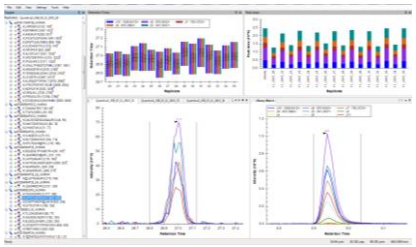
Run SRM Method

Evaluate SRM Results

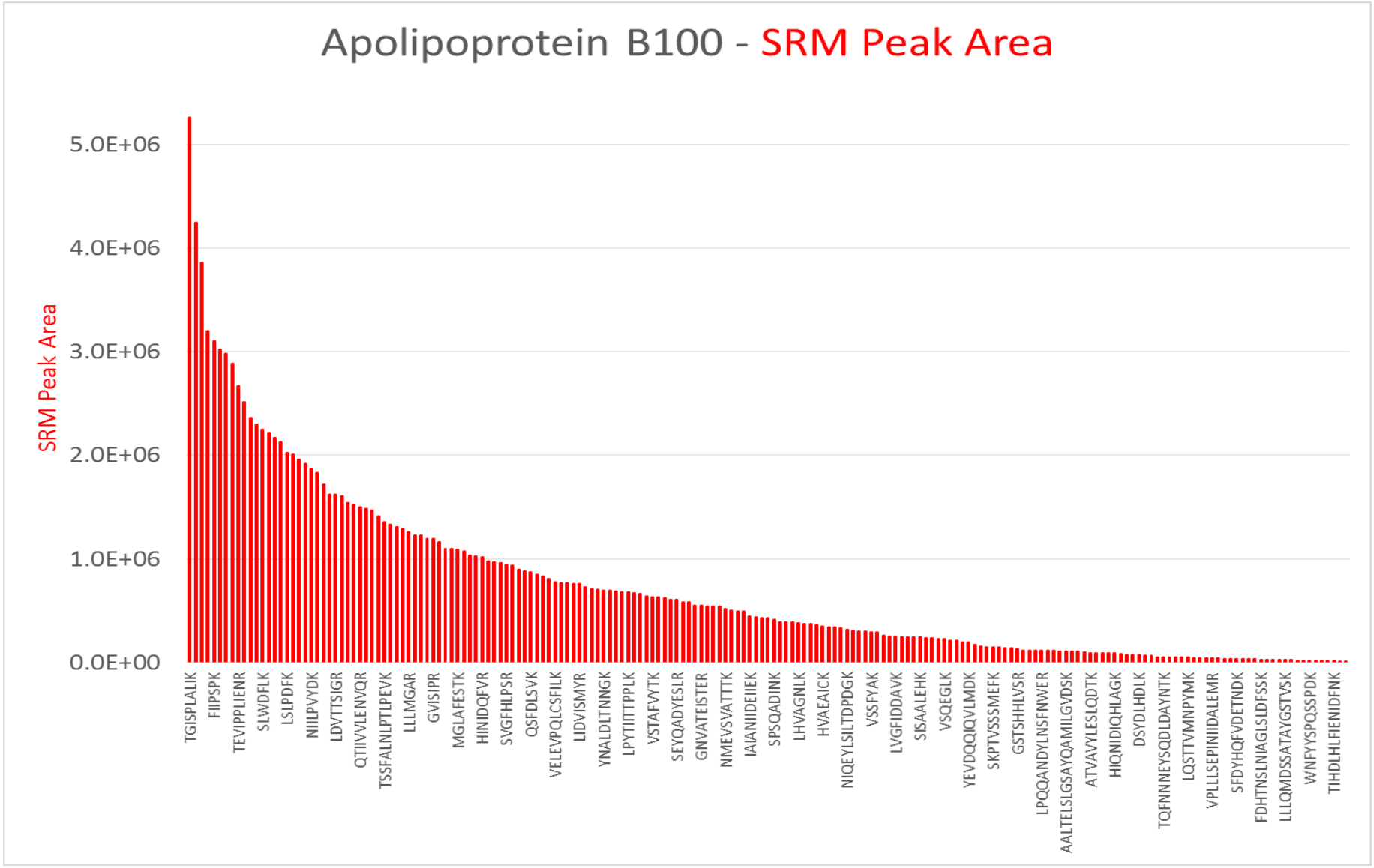
Peptide Refinement Criterion:

- Intensity
- Product Ion Distribution
- Stability
- iRT Calibration
- Detectability in Matrix
- Digestion Kinetics
- Precision
- Accuracy

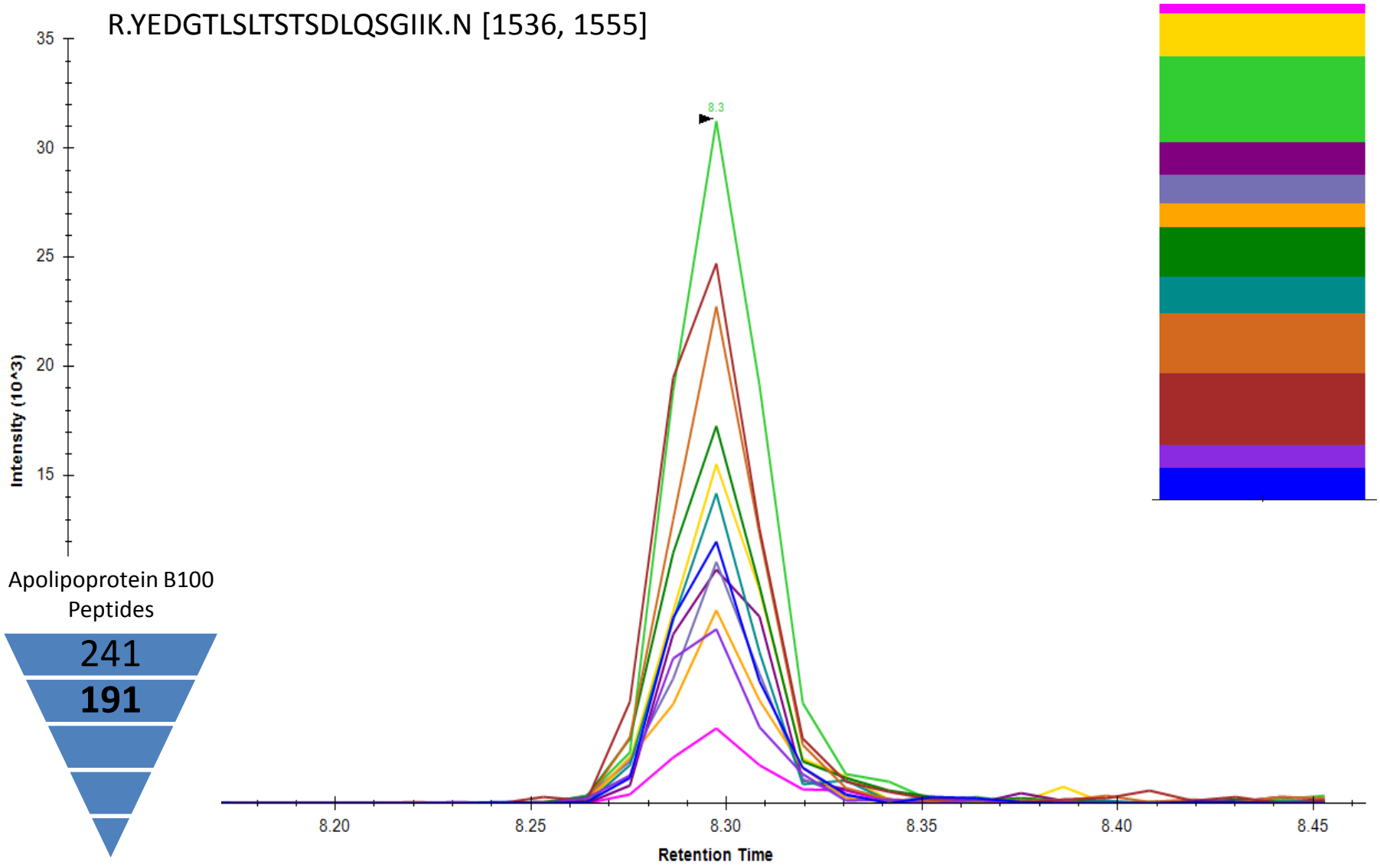
Skyline



Round 1 nanoLC MS/MS: SRM Signal Intensity Rank



Round 1 nanoLC MS/MS: Relative Product Ion Distribution

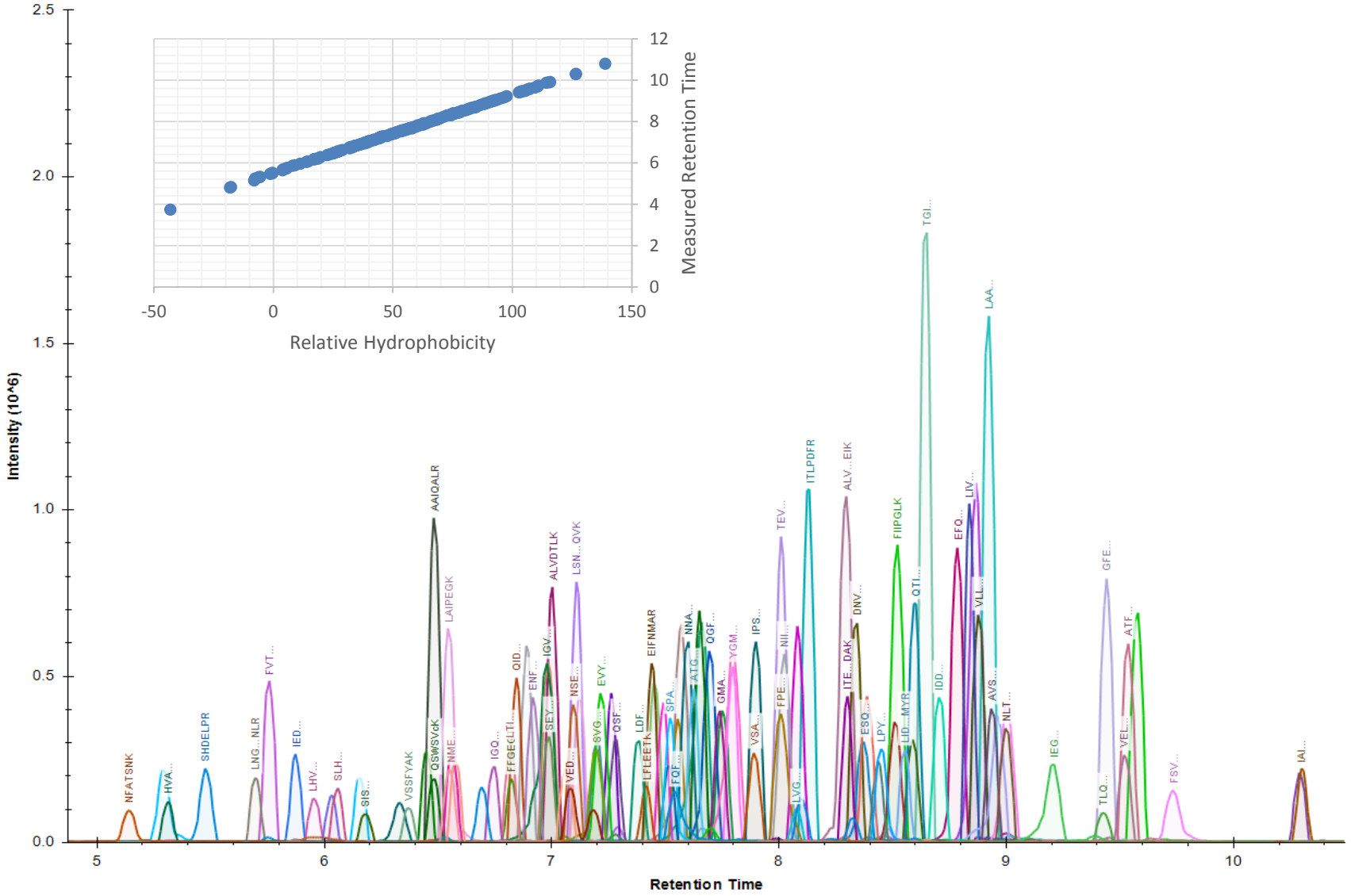


Apolipoprotein B100 Peptides

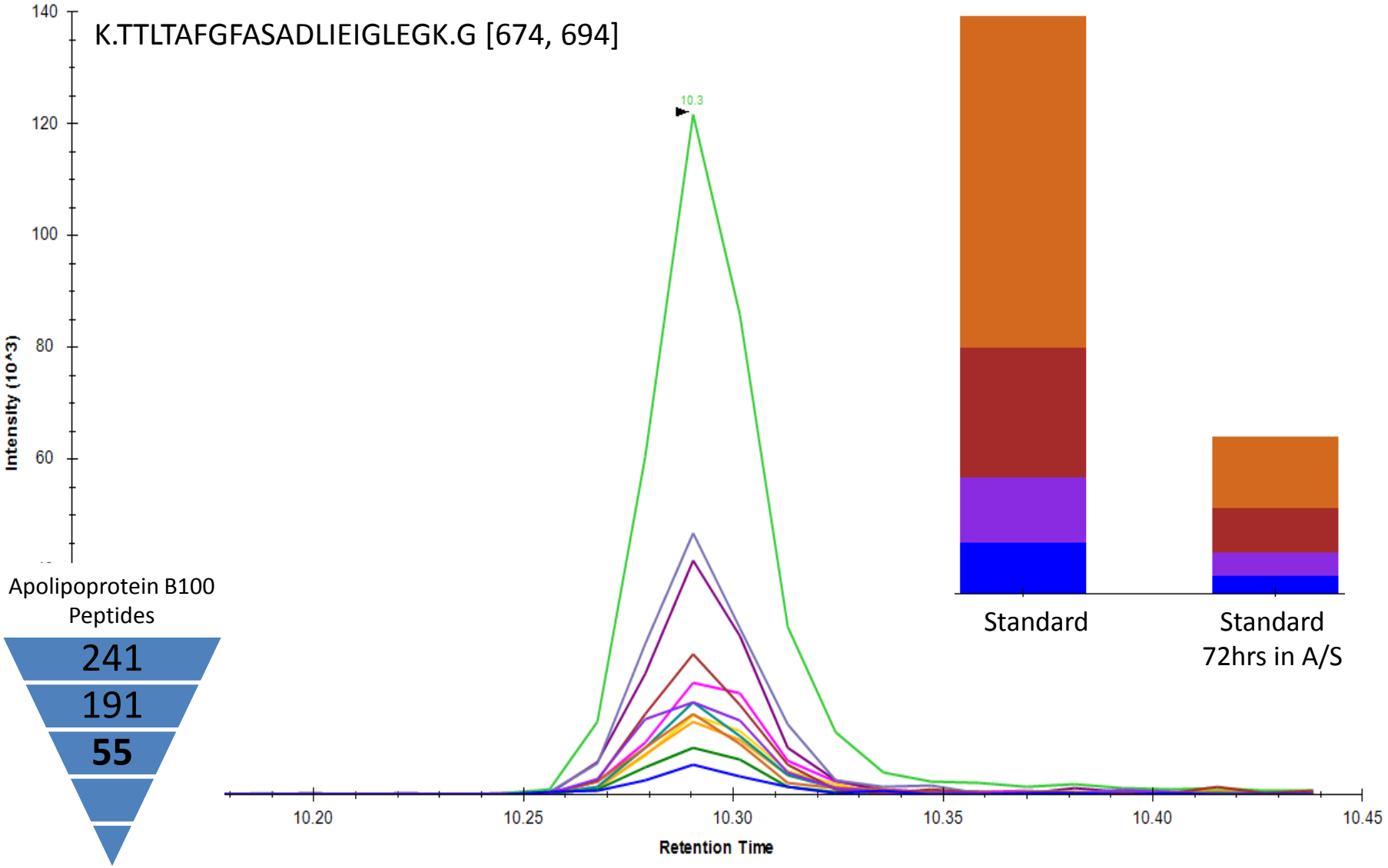
241

191

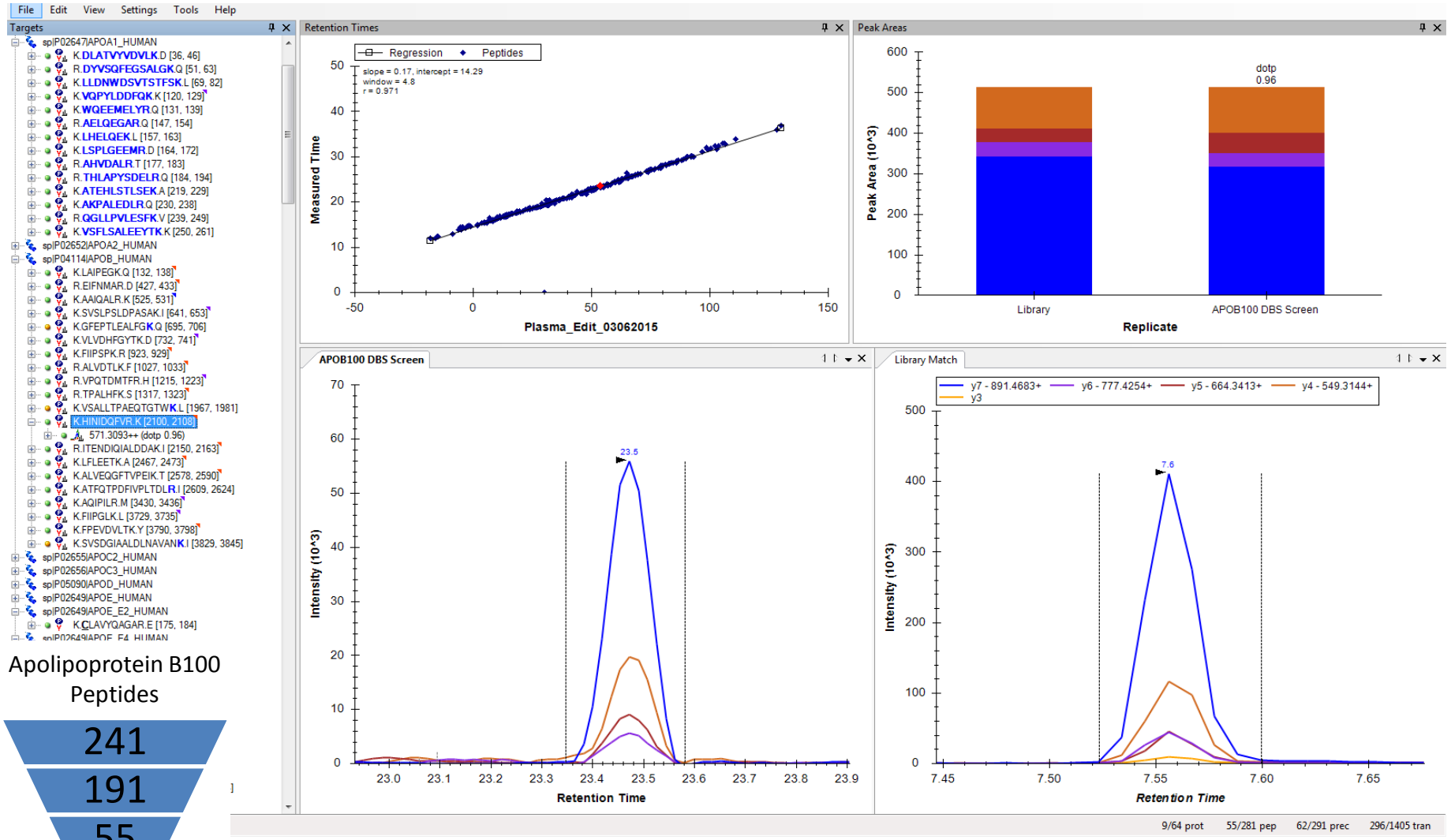
Round 2 nanoLC MS/MS: iRT Calibration with ^{15}N-APOA1



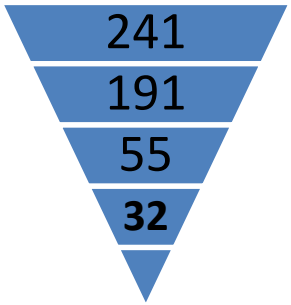
Round 2 nanoLC MS/MS: Peptide Stability Analysis



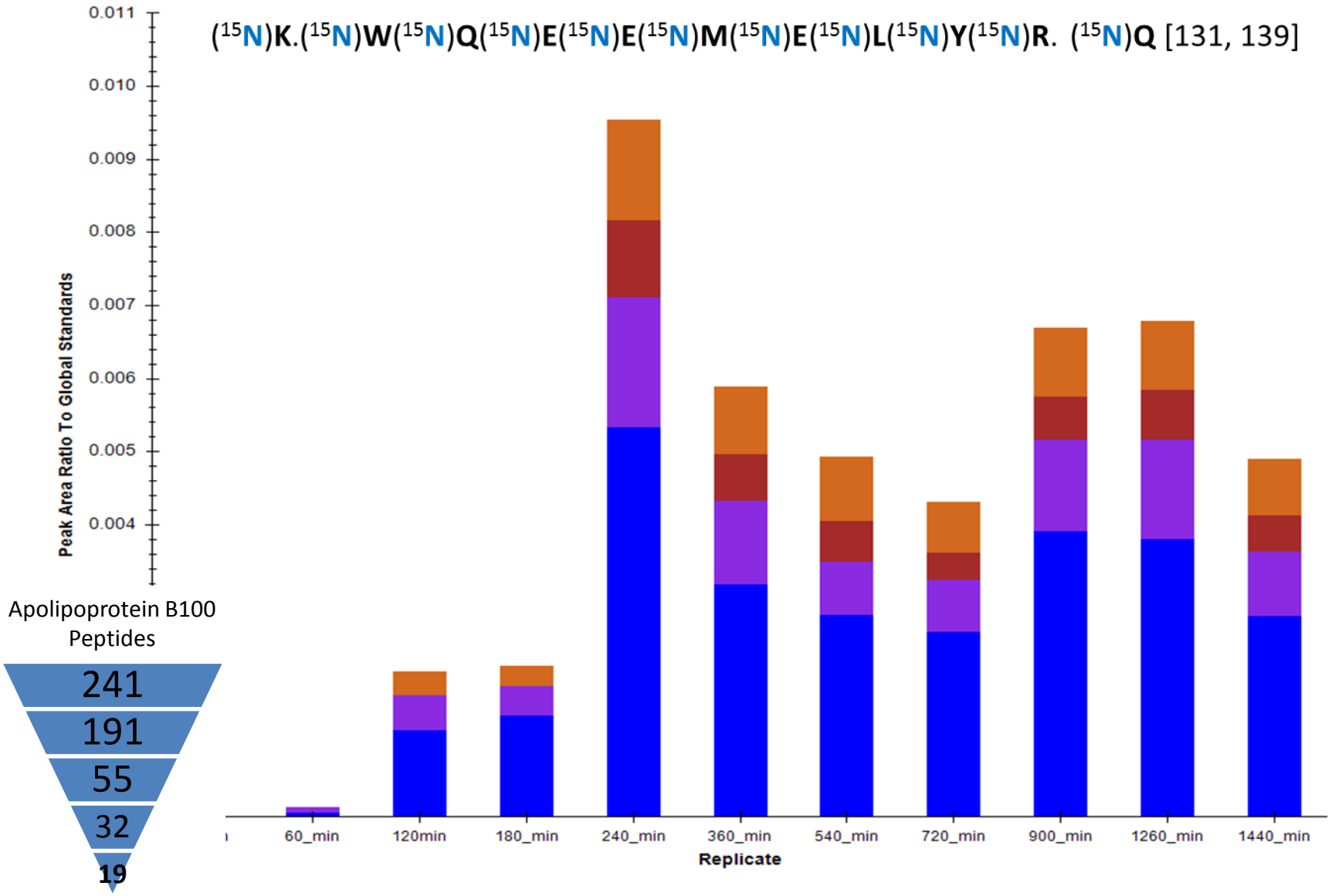
Round 3 nanoLC MS/MS: Validation of Peptides/Transitions in Matrix



Apolipoprotein B100 Peptides



Round 4 nanoLC MS/MS: Digestion Time Course



Apolipoprotein B100 Peptides

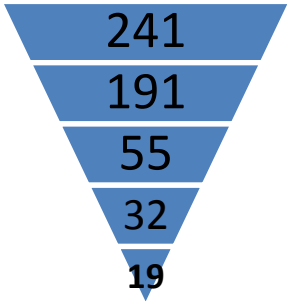
241
191
55
32
19

Method Refinement Considerations

Empirical Peptide Refinement:

Round 1 -> peptides with non-existent or ambiguous chromatograms
Round 2 -> peptides that fail to meet minimum stability requirements
Round 3 -> peptides that were not observed in matrix
Round 4 -> peptides that gave a sub-optimal digestion characteristics

Apolipoprotein B100 Peptides



Normalization?

APOA1 Global Internal Standard (^{15}N Protein)[1]

Table 1. Performance characteristics of a multiplexed LC-MRM/MS assay.

	IS_{pep}[a]					IS_{prot}[b]				
		Imprecision, % CV					Imprecision, % CV			
	r[c]	Linearity[d]	LC-MS[e]	Digest[f]	Total[g]	r	Linearity	LC-MS	Digest	Total
A-I	0.79	0.9982	2.6	9.4	9.8	0.96	0.9994	5.6	2.3	6.1
B	0.67	0.9983	6.4	2.6	6.9	0.61	0.9975	8.9	3.5	9.6
C-II	0.90	0.9948	4.1	4.6	6.1	0.92	0.9937	6.1	4.1	7.4
C-III	0.89	0.9962	9.5	5.0	10.7	0.88	0.9992	11.8	4.8	12.8
E	0.96	0.9984	2.2	3.3	4.0	0.92	0.9984	2.2	2.1	3.1
J	0.81	0.9994	9.3	6.8	11.5	0.79	0.9991	12.4	3.3	12.8

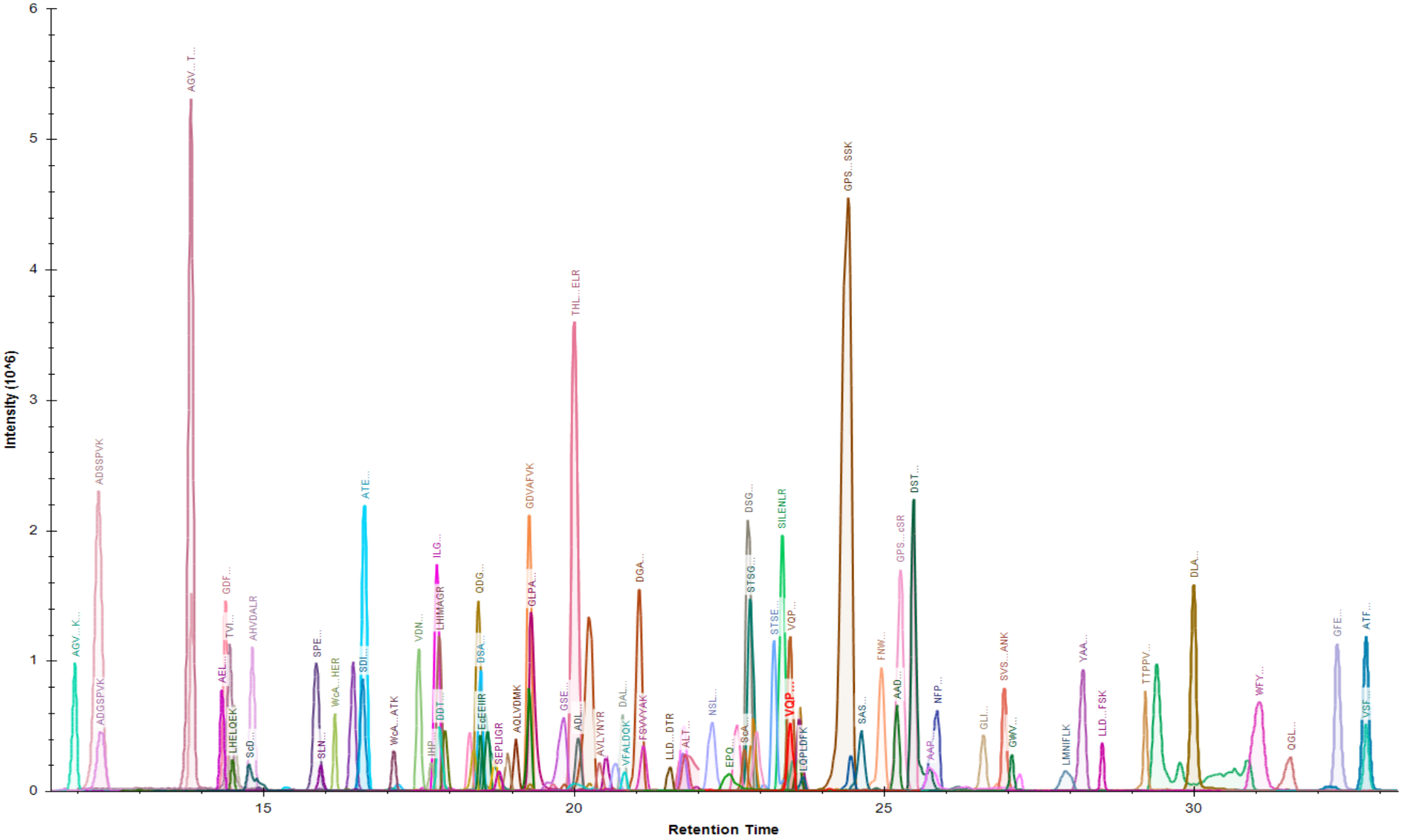
1) Hoofnagle, A.N. et al. *Clin. Chem.* **2012**, 58(4); 777-781.

Calibration?

Single Point Calibrator[2] - Assign concentration with clinical immunoassay & measure in triplicate in each batch

2) Cox, B. et al. *Clin. Chem.* **2014**, 60(3); 541-8.

Scheduled SRM Method for Analysis of DBS



Interested in developing a similar targeted assay?

- Let the MacCoss lab help!
- Services and consulting offered for development and application of targeted proteomics assays:
  - DIA
  - PRM
  - SRM
- Website: **http://services.maccosslab.org**
- Email: services@maccosslab.org
- In Person: Jarrett Egertson or Mike MacCoss